IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	SANDERS et al.	Group Art Unit:	1648
Serial No.:	10/516,578	Examiner:	Bo Peng
Filed:	16 November 2005	Confirmation No.:	5513
For:	PSEUDOTYPED RETROVIRUSES		

DECLARATION OF DAVID A. SANDERS UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, David A. Sanders, declare and say as follows:

1. I received a Ph.D. from University of California – Berkeley in Biochemistry in 1989; and a B.S. in Molecular Biophysics and Biochemistry from Yale College in 1983.

2. From 1989 to 1990, I worked as a Postdoctoral Fellow at University of California – San Francisco (UCSF), where I studied the structure and molecular mechanisms of GTPases. From 1990 to 1995, I worked as a Postdoctoral Fellow at Whitehead Institute for Biomedical Research in Cambridge, MA, where I studied the impact of viral envelope proteins and pseudotying of viruses on retroviral entry. From 1995 to 2001, I worked as an Assistant Professor in the Department of Biological Sciences at Purdue University in West Lafayette, IN, where I studied viral envelope protein complexes and pseudotyped viruses.

3. Since 2001, I have been employed as an Associate Professor in the Department of Biological Sciences at Purdue University in West Lafayette, IN. My laboratory continues to study a variety of viral envelope proteins, including retroviral proteins, and their role in viral entry. We also study the potential of utilizing viral vectors for gene therapy.

4. I have read and am familiar with the Office Action dated November 25, 2009, and Yang et al. (*Nature Medicine*, 2000, 6(8):886-889) and Simmons et al. (*J. Virology*, 76(5):2518-2582, 2002) cited therein.

5. Yang et al. (2000) describe a mutant Ebola glycoprotein, GP(Δmuc). Yang et al. do not define the deleted region by any start and stop residues, nor do Yang et al. teach what portion of the Ebola GP sequence is missing. Yang et al. used the amino acid sequence cited in the Office Action, amino acids 315-505, as an insert for envelope protein 4070A of MLV and subcloned amino acids 315-505 into an expression vector (Yang et al. at page 889, second col.). Yang et al. do not reference this sequence in connection with the GP1 *deletion*.

6. Yang et al. investigated the pseudotyping ability of their deletion mutants, including the undefined GP(Δmuc), using a green fluorescent protein [GFP] reporter assay. A murine leukemia virus hybrid retroviral vector expressing GFP was used to generate the pseudotyped retroviral vector. Human endothelial cells were infected with the pseudotyped viruses, and GFP expression was measured. On the basis of this assay (Fig. 1b), Yang et al. concluded that GP(Δmuc) was "expressed and functionally active at levels similar to those of wild-type GP" (Yang et al. at page 886, second col.). Yang et al. do not describe increased titer levels or transduction efficiency for the pseudotyped retroviruses.

7. Simmons et al. teach a murine leukemia virus pseudotyped with an Ebola GP that contains a deletion in the mucin-like domain of GP1 (Simmons et al., page 2519).

Several mutants are taught, including one, mutΔ1234, which has a deletion between amino acid 311 and 463 (Simmons, Fig. 1A) and which lacks all of the predicted C-terminal O-linked glycosylation sites (Simmons et al., page 2520 bridging to page 2521).

8. Simmons et al. found that glycoprotein expression (measured as GP2 levels) was about the same for the mutants as well as the wild-type GP (see Fig. 1B which shows GP expression in mutΔ1234 at 136% of wild-type GP). Coexpression of mutΔ1234 with plasmids encoding MLV Gag/Pol and LacZ produced infectious MLV pseudotype particles with titers equivalent to those of wild-type EboZ GP (Simons et al., page 2520 bridging to page 2521). Simmons et al. do not describe increased transduction efficiency for any of the GP mutants.

9. Both Yang et al. and Simmons et al. represent attempts to elucidate the determinants of Ebola's vascular cytotoxicity and injury, and both use deletion mutants to explore the effects on cell morphology, cell death, and the like. However, neither study found that deletion of the mucin domain had a major effect on Ebola glycoprotein-pseudotyped virus production.

10. A pseudotyped retrovirus that contains an Ebola glycoprotein containing a deletion of an amino acid sequence encoded by codons 309 to 489 of SEQ ID NO:1, as recited in claim 1, is described in Jeffers et al., *J. Virology* 76(24):12463-12472, 2002, of which I am a co-author. This paper also describes a number of other mutations within the Ebola virus glycoproteins. Table 3 of Jeffers et al. (2002) is reproduced below and shows that the other mutations failed to significantly increase transduction titers. In fact, a number of analyzed mutants show a decrease in transduction titers. As evidenced by the data in the table, the significantly increased transduction titers associated with the Ebola Δ309-489 GP construct were not predictable.

Table 3. Transduction of NIH 3T3 cells by virus pseudotyped with mutant Ebola virus GPs with altered glycosylation

Mutant GP	% Transduction[a]
N40D	<0.1
T42D	113 ± 17
N204D	102 ± 14
N238Y	88 ± 4
N257D	88 ± 9
N277D	84 ± 10
N296D	62 ± 10
N563D	80 ± 4
N618D	102 ± 3
Δ309-489	696 ± 142

11. Increased expression of Ebola glycoprotein in retroviruses pseudotyped with the Ebola Δ309-489 GP is shown in Figure 7 of Jeffers et al. (2002). As expected, the Ebola Δ309-489 GP migrates faster due to the smaller size resulting from the deletion. However, in view of the fact that equal amounts of each sample were used in each lane of the SDS-PAGE (Jeffers et al. (2002); page 12465, left-hand column), the increased density of the Ebola Δ309-489 GP protein shows increased expression of the Ebola Δ309-489 GP.

12. Jeffers et al. thus shows that the deletion of codons 309 to 489 of the Ebola glycoprotein led to a striking and unexpected increase in both expression of Ebola glycoprotein and transduction efficiency of a retrovirus pseudotyped with this glycoprotein.

13. Increased transduction efficiency of a retrovirus pseudotyped with Ebola Δ309-489 GP is also shown in Sanders et al. (*Exp. Opin. Biol. Ther.*, 4:329-336, 2004), a peer-reviewed review article that I authored. Page 333 states that "[r]emarkably, deletion of virtually all of this region of *O*-glycosylation resulted in the enhancement of Δ309-489 GP processing and incorporation into recombinant MuLV. Consequently, dramatically higher transduction titers were obtained." In addition, the review article reports that feline

immunodeficiency virus (FIV), a lentivirus, pseudotyped with Ebola Δ309-489 GP demonstrated a striking 74-fold increase in transduction compared to FIV pseudotyped with wild-type Ebola glycoprotein.

14. Retroviruses pseudotyped with the Ebola Δ309-489 glycoprotein exhibited expression levels and transductions efficiencies that together were dramatically higher than those reported for retroviruses pseudotyped with other mutant Ebola glycoproteins. These results were unexpected and unpredictable.

15. I declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

March 24 2010 By: David A. Sanders

Date David A. Sanders